

THE SUBMICROSCOPIC STRUCTURE OF THE
ORAL MUCOSA OF THE PHALANGER
(*TRICHOSURUS VULPECULA*)*

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(Plates III-VIII)

[Read 26th March, 1969]

Synopsis

The structural pattern of the oral mucosa of the phalanger, *Trichosurus vulpecula*, is investigated, and compared with that of the oral mucosa of the ox, *Bos taurus*, and of the finch, *Steganopleura annulata*.

It is found that in the oral mucosa of the phalanger the intracellular fibrillous net develops stronger than in ox. Within the cell the net is distributed peripherally, a condition also known in some birds. The characteristics of phalanger's oral mucosa is an extensive incorporation of cytoplasm into the fibrillous bundles as well as appearance of spacious enlargements of the intercellular spaces.

The similarities between the proteinaceous bodies in plants and protozoans to the tubular structure of nets in phalanger are discussed.

INTRODUCTION

The fine structure of the oral mucosa has been investigated in a limited number of species only and no report on its morphology in marsupials is known to me. Consequently our previous studies on the oral mucosa (Tucker 1966, 1968) were extended to the phalanger (*Trichosurus vulpecula*) in an attempt to obtain additional comparative data. As the mechanism of the food intake is a main source of forces acting on oral mucosa it is of interest to note that in eating the phalanger nibbles constantly and as a marsupial it feeds itself orally at a much earlier stage than do the placental mammals. In addition, marsupials in the pouch initially attach themselves to a teat, so this could act on and modify the oral mucosa of a marsupial to a greater extent than is the case with placental mammals.

MATERIAL AND METHODS

Portions of the oral mucosa of the phalanger (*Trichosurus vulpecula*) from the vicinity of the frenulum were collected under ether anaesthesia, immediately fixed in 4% glutaraldehyde at 4°C. for 2 hours, washed overnight in 2% sucrose, buffered with sodium cacodylate (pH 7.3), and then post-fixed in 1% osmium tetroxide, dehydrated in alcohol, and embedded in epon. The sections were stained on the grid with 5% uranyl acetate and then with lead citrate. The sections were viewed on a Siemens Elmiskop I.

RESULTS

The composition of the oral mucosa

The cells of the oral mucosa of the phalanger are uniform and have large, generally chromatin-poor nuclei (Plate III, figs 1 and 2) with marginally-distributed chromatin and a prominent nucleolus (Plate III,

* Written on the occasion of Professor H. Grau's anniversary.



fig. 2). Also shallow invaginations of the nuclear membrane were often observed (Plate III, figs 1 and 2). The distribution of the cell organelles is a characteristic one—the nucleus lies centrally, the fibrillous net peripherally, and between them spreads out a relatively clear cytoplasm with single or cluster-like granules in it. (Plate III, figs 1 and 2). In the peripheral portion of the cell (Plate III, fig. 1), are present strong bundles of fibres, broad and ribbon-like, which join each other, spreading out through the marginal cytoplasm as a common cellular net (Plate III, fig. 3, Plate IV, figs 4 and 5). The marginal condensations of fibres produce numerous and powerful desmosomes (Plate IV, fig. 6, Plate V, fig. 7) each of which is firmly attached to a fibrillar bundle, and through it to the intracellular fibrillous net (Plate V, figs 8, 9, 10 and 11).

The transverse sections through desmosomes and through the fibrillous ribbons have shown that the light areas which give them at times a doughnut-like appearance are in fact portions of the cytoplasm incorporated into the bundle. Smaller cytoplasmic accumulations, trapped between and along the fibrils, give the impression of a whole bundle being tube-like in structure (Plate VI, figs 12, 13 and 14).

In the proximity of fibrillous bundles and desmosomes are present irregular aggregations of single fibrils which in transverse sections simulate the granules (Plate VI, fig. 15).

The intercellular spaces produce (according to the section) a considerable number of uniformly spherical, large, and evenly-spaced intracellular sinuses (Plate VII, figs 16 and 17) and much more seldom large and irregular enlargements (Plate VII, fig. 18).

In the intercellular spaces of the mechanically active mucosal cells, microvilli were observed only in a few isolated instances. They are however well developed around the secretory cells (see below). The intercellular fluid contains some dark granules and at the level of the desmosomes it condenses to form 3 distinct bands, parallel to the cell surfaces (Plate VII, fig. 19). The intercellular sinuses of the phalanger's mucosa are flanked with thick fibrillous bundles and bulky desmosomes (Plate VII, figs 20 and 21, Plate VIII, fig. 22). The remnants of a substance of varying electronic density were traced in many sinuses. Also in a few cases very regular cytoplasmic spheres have been seen (Plate VIII, fig. 23). This suggests the existence of cellular interdigitations.

In the oral mucosa of the phalanger a number of goblet cells were observed. These cells, although usually single, tend here to accumulate in a circumscribed area (Plate VIII, fig. 24). In the early secretory stages they possess some endoplasmic reticulum and the intercellular spaces between them are crowded with microvilli (Plate VIII, fig. 25).

The nuclei of the goblet cells possess more chromatin than the epithelial cells of the oral mucosa. In the cytoplasm of goblet cells are placed small elongated mitochondria as well as large areas of granules. The secretory droplets are round in sections and their growth leads to the compression of the cytoplasm and consequently to the increase of its electronic density. Capillaries and the large accumulations of collagen fibres are present in the proximity of the goblet cells.

DISCUSSION

Our previous researches on the epithelial cells of the oral cavity (Tucker, 1966, 1968) have shown extensive development of fibrillous proteins which perform mechanical tasks. In this respect the oral mucosa of phalanger

shows, 1. A greater development of mechanical organelles: fibrils, desmosomes and nets, 2. a tubular arrangement of protein not seen in other mammals and 3. a pronounced incorporation of cytoplasm into the tubular or fibrillous nets, a property which is only slightly indicated in other mammals.

In this respect it is of interest to note that proteinaceous bodies of fibrils, and tubular structures resembling those in the oral mucosa of the phalanger have been described in some plants—*Nicotiana tabacum*, *Pisum sativum*, *Phaseolus vulgaris* and *Cucurbita maxima* (Cronshaw and Esau, 1968). According to the shape of the proteins they are described as P. 1, 2, 3, or 4 bodies. The incorporation of cytoplasm into proteinaceous nets in the phalanger bears some similarity to the incorporation of cytoplasm by P. 3 proteinaceous bodies of *Cucurbita*, and in some places by P. 4 type bodies.

In plants and mammals the accumulations of proteins are formed without any limiting membrane around them and they push away all other organelles of the cell (Tucker 1966, 1968; Cronshaw and Esau, 1968). Cronshaw and Esau outlined the development of proteinaceous bodies in *Cucurbita*. No detailed study of the ontogeny of fibrillous nets in mammals has yet been undertaken but our previous comparative studies (Tucker 1966, 1968) indicate that they originate from single fibrils on the peripheral portion of the cytoplasm.

The formation of mechanical organelles observed in mammalian epithelial cells is probably a very general property of cells as fibrillar organization of a similar type has been reported for the giant Amoeba *Chaos chaos* (Nachmias, V. T. 1968).

A particularly striking feature of the oral mucosa of the phalanger is the extreme development of the intracellular fibrillous net. The fibrillous net is more regular than that in the oral mucosa of the ox and the fibrillous bundles in the net, including desmosomes, achieve much greater strength than those in the ox.

The volume of fibrillous bundles and the frequently perpendicular position of them to each other made it possible to see the laterally irregular spreading out of fibrils and the engulfing of cytoplasm into the bundle (Plate VI, figs 12, 13 and 14). This may explain the presence of the doughnut-like desmosomes described earlier in the ox (Tucker, 1966) as well as the internal structure of the fibrillous bundles, which in transverse sections may appear as a set of small tubules. As the cytoplasm incorporated into the bundle has a relatively small electronic density, the whole arrangement can be considered to be a repetition of the relationship between the intercellular sinuses and the strong fibrillous elements in so far as the areas of small density are surrounded by the dense fibrillous elements. Also the intracellular sinuses and the enlargements of the intercellular spaces are larger than in the ox and their structure is simpler. Microvilli are seldom seen. The enormous development of the fibrillous bundles and the size of enlargements of the intercellular spaces suggests that in phalanger the exchange of energy between those systems is efficient. Further, such a transition of energy is probably connected with a considerable magnitude, or with a considerable frequency of stresses or both. It may be due to an early intake of the solid food.

In addition, the lips of a young in the pouch can be used as an attachment organ to the teat.

With reference to the differences between the intercellular spaces around the goblet cells and those around the mechanically-working cells it can be noted that such enlargements of the intercellular space which contain

villi have also probably a greater stress dispersing ability than the simple enlargements of slits (Tucker, 1966). The above supposition is corroborated by the lesser rigidity of the goblet cells than that of the fibrillous cells of the oral mucosa. Further, in the case of the sudden collapse of a goblet cell during normal secretory activity, the presence of microvilli may make it easier to preserve the intercellular spaces because of the resistance of the microvilli to pressures from the surrounding cells (Text figs 1 and 2).

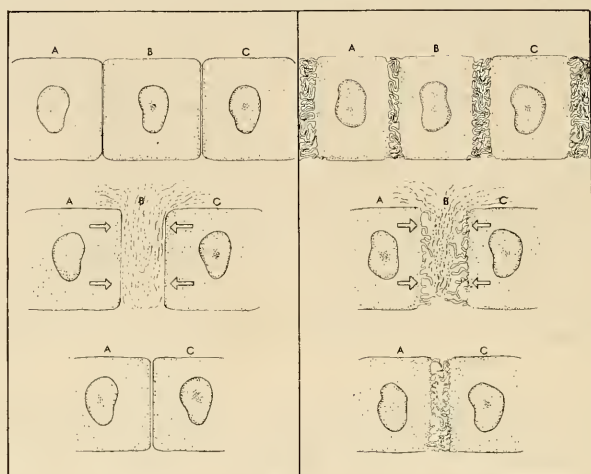


Fig. 1.

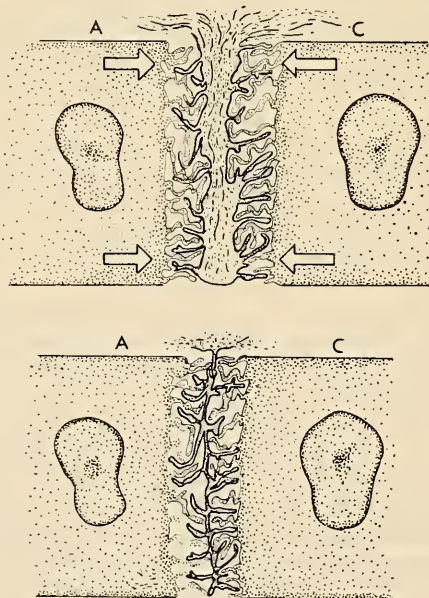


Fig. 2.

In the oral mucosa of ox (Tucker, 1966, 1968) the enlargements of the intercellular spaces have usually a complex morphology with microvilli while in phalanger the corresponding enlargements are simple, (without microvilli). This is accompanied by the augmentation of the fibrillous net in the cells of the oral mucosa of phalanger (as compared with that in the ox). The predominant distribution of fibrils on the periphery of the epithelial cells

resembles conditions in some birds (Tucker 1968). Also in finch (*Steganopleura annulata*) the intercellular processes of the epithelial cells interdigitate causing a tortuous outline of the intercellular spaces while in phalanger the processes of 2 cells seem to be often at the same level resulting in a series of the bulbous enlargements of the intercellular space (Text fig. 3). In the phalanger the cytoplasm may be incorporated into the fibrillar bundles along the whole length of the bundle, while in ox this was observed only at the base of fibrillous condensations (Tucker, 1966).

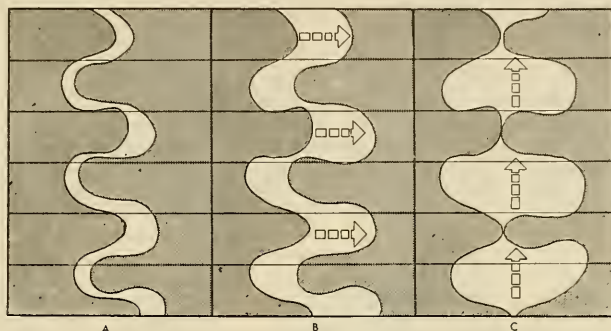


Fig. 3.

Acknowledgements

It is the author's pleasure to acknowledge the financial support received from the Rural Credits Development Fund of Australia and the help of Mrs. L. Endean in preparation of the manuscript. Mr. D. Gowanlock and Miss A. Robinson of the Electron Microscope Unit contributed to the preparation and manipulation of the material. My thanks are also due to Mr. Hardy of the above unit for his interest in this investigation.

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EXPLANATION OF PLATES

- PLATE III. 1. $\times 4,000$, shows the general composition of the oral mucosa.
 2. $\times 20,000$, shows the nucleus of a mucosal cell with a nucleolus and peripheral nuclear invaginations.
 3. $\times 46,000$, shows the composition of the fibrillous net.
 PLATE IV. 4. $\times 72,000$, shows the orientation of fibre bundles, running perpendicularly to one another, in the fibrillous net.
 5. $\times 64,000$, shows ribbon formation in the net.
 6. $\times 60,000$, shows a large desmosome.
 PLATE V. 7. $\times 56,000$, shows marginal doughnut-like condensations and desmosomes.
 8. $\times 40,000$, shows a desmosome and its attachment to the fibrillous net.
 9. $\times 36,000$, shows a desmosome and its attachment to the fibrillous net.
 10. $\times 16,000$, shows a desmosome and its attachment to the fibrillous net.
 PLATE VI. 11. $\times 20,000$, shows a desmosome and its attachment to the fibrillous net.
 12. $\times 60,000$, shows a transverse section through the fibrillous complex.
 13. $\times 56,000$, shows cytoplasmic inclusions in the fibrillous condensations.
 14. $\times 50,000$, shows the engulfing of the cytoplasm by the fibres.
 15. $\times 60,000$, shows the granules in the cytoplasm.

PLATE VII 16. $\times 20,000$, shows the distribution of intercellular spaces.

17. $\times 20,000$, shows the distribution of intracellular sinuses.

18. $\times 32,000$, shows a large, irregular enlargement of an intercellular space.

19. $\times 80,000$, shows parallel lines in the intercellular space on the level of desmosomes.

20. $\times 20,000$

21. $\times 36,000$

PLATE VIII. 22. $\times 20,000$ } show the close relationship between inter-

cellular spaces and fibrillous bundles.

23. $\times 16,000$, shows intracellular sinuses containing interdigitating processes or

remnants of cytoplasmic material.

24. $\times 4,000$, shows the accumulation of secretory cells and the reduction of cytoplasm.

25. $\times 20,000$, shows an intercellular space with microvilli between secretory cells in the oral mucosa of the phalanger.

EXPLANATION OF TEXT FIGURES

Fig. 1. Diagram illustrating the possible influence of microvilli on the maintenance of the intercellular space. In the left-hand column the cell B is shown undergoing holocrine disintegration. The stresses from the surrounding tissue push the cells A and C together, closing completely the space between them. Note that none of the cells A, B, or C has microvilli, and that consequently the maintenance of the intercellular space is very difficult.

In the right-hand column the general situation is repeated, except that cells A, B and C have microvilli. In consequence, even if the cell B is completely destroyed, the microvilli of cells A and C still facilitate the maintenance of the intercellular space.

Fig. 2. Shows the reduction of the middle cell in such a way that the cytoplasm pours out, but the cellular membrane remains in situ. In such a case, the remnants of this cell may facilitate the initial stages of formation of a new intercellular space.

Fig. 3. A diagram illustrating the difference in sequences of the cellular processes in the oral mucosa.

A represents the condition in birds, B shows an intermediate position often seen in the oral mucosa of the ox, and C illustrates the processes in a new relative position, and formation of the larger intercellular spaces as seen in the phalanger.